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IN VITRO EFFICACY OF BIOPESTICIDE (*BEAUVERIA BASSIANA*, *METARHIZIUM ANISOPLIAE*, *BACILLUS THURINGIENSIS*) AGAINST MUSTARD APHID *LIPAPHIS ERYSIMI* KALT. (HEMIPTERA: APHIDIDAE

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ABSTRACT

Brassica crop is ranked 2nd most important oil seed crop in Pakistan and rich source of oil and protein. The lowest level of erucic acid and glucosinolates are the preferred characters of *Brassica* oil crop. In addition, its meal has 38-40% protein content which has complete profile of amino acid like lysin, methonin and cystin. Mustard aphid, *Lipaphis erysimi* Kalt. (Homoptera: Aphididae) is the limiting factor of qualitative and quantitative losses by attacking and hurting the leaves and pods in growing area of Pakistan and also develop resistance to some synthetic insecticides. Biopesticides are target specific, retard insect growth, metabolic process and has no adverse toxicity to mammals. Present study was planned for comparative efficacy of three biopesticides (*Beauveria bassiana, Metarhizium anisopliae* and *Bacillus thuringiensis*) in vitro with five concentrations and three replications of each insecticide. The complete randomize design was used. All biopesticides were use at 5, 10, 15, 20 and 25% concentration. The percent mortality at highest concentration of *B. bassiana*, *M.* anisopliae and *B. thuringiensis* was 78%, 83% and 73%, respectively after three days of application. Among the biopesticides *M. anisopliae* was found most effective against mustard aphid followed by *B. bassiana* and *B. thuringiensis*. *M. anisopliae* could be used as potential candidate for integrated pest management against mustard aphid after field efficacy.

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INTRODUCTION

Insect pest is an important qualitative and quantitative yield limiting factors of *Brassica juncea* (Singh, 2010). Mustard and rape seed crops are attacked by a number of sucking insect pests including aphid, whitefly, mustard sawfly, green bug, painted bug and chewing insect including cabbage butterfly, armyworm, looper, hairy caterpillar, diamond back moth, pea leaf miner and cricket (Hainan et al., 2007). Nearly 92 species of aphid are found in Pakistan and most of them are important insect pests of crucifer crops, vegetables and fruit trees (Irshad, 2001). Mustard aphid, *Lipaphis erysimi* Kalt. (Homoptera: Aphididae) has been reported as a dominant pest in districts of southern Panjab, Pakistan (Farooq, 2007; Sarwar, 2013). *L. erysimi* is responsible for 9 to 96% yield losses (Singh and Sharma, 2002) and reduces 15% oil contents (Verma and Singh, 1987). *L. erysimi* is a destructive pest of *B. napus*. Both adult and nymphs cause damage to plant in two different ways (Bak et al., 2013) directly by sucking the sap from the phloem of the plant during vegetative stage to seed setting stage (Louis and Shah, 2013) and indirectly by aphid cause the losses of

plant by secreting honeydew on which black sooty mould grows that reduces the plant vigour and photosynthetic area of leaf (Sarwar, 2013). Due to parthenogenetic reproduction and short generation times, aphid population increases rapidly (Hales et al., 1997) and damage percentage also. Aphid also acts as vector for various viral diseases transmission (Sarwar, 2013) which causes the deformation of pods, flowering buds, leave curling at pod formation, flowering and vegetative stage of plants (Opfer and McGranth, 2013).

Flowering stage at the risk of phloem sap sucking *L. erysimi* (Singh, 2010), April to November is the peak period and affected by Sunstroke and relative humidity (Francisco and Santos-Cividanes, 2010). The control of mustard aphid is inevitable through foliar spray, soil application and seed treatment with insecticides all over the world (Gogi et al., 2006; Sarwar, 2013). To control the aphid, growers of *Brassica* crops blindly use conventional insecticides which posed several ecological changes like resistance development, bio control agent's equilibrium disturbance, environmental pollution and accumulation of toxic substances in food commodities that lead to health hazards like cancer, kidney and liver failure and genetic disorders in human beings (Ambethgar, 2009; Owain et al., 2008). These issues can be overcome by developing safe and eco-friendly management approaches.

Living organisms are the source of biopesticides and microorganisms are the active ingredients. Extraction process does not alter the chemical composition of microorganisms (Lee et al., 2000). Biopesticides have been potentially used against different agricultural insect pest (Fargues, 1975; Hall, 1963). Biopesticides are world-wide available commercially with different formulations and brand names (Faria and Wraight, 2007).

Keeping in view these facts, present research was conducted to assess the individual performance of bio pesticides i.e. *Beauveria bassiana*, *Metarhizium anisopliae* and *Bacillus thuringiensis* against *L. erysimi* with the objective to find out that biopesticides and IGRs are the best alternatives to conventional insecticides.

MATERIALS AND METHODS

Insect collection: Mustard aphid, *Lipaphis erysimi*, was collected from brassica fields. Aphid was placed in ventilated plastic jars and brassica leaves were used as food for aphids. The aphids were checked for disease and parasitism and only healthy individuals were used in pathogenicity assays.

Insecticides: The biopesticides and insect growth regulators (IGRs) used in the research are given in Table 1.

Concentration preparation: Five conidial suspensions (dilutions) *i.*e., 5, 10, 15, 20 and 25% of each biopesticides were prepared. The determined quantity of each was mixed in water up to required volume to prepare 5, 10, 15, 20 and 25% dilutions. The colony forming units (CFU) were counted by using potato dextrose agar (PDA) medium or hemocytometer.

Table 1. The biopesticides and insect growth regulators (IGRs) used in the experiments.

Active ingredient	Trade name	Formulation	Category
Bacillus thuringiensis var kurstaki	Lipel ®	Wettable Powder	Biopesticide
Beauveria bassiana	Racer ™	Wettable Powder	Biopesticide
Metarhizium anisopliae	Pacer ®	Spray able Powder	Biopesticide

Calculation of colony forming unit of bacteria and fungi: Colony-forming unit is a measure of viable bacterial or fungal cells. Serial dilutions, plating and counting of live bacteria was used to determine the number of bacteria and fungi in a given population. Serial dilutions of a solution were made for containing an unknown number of bacteria and fungi, plated these bacteria and fungi and determined the total number of bacteria and fungi in the original solution by counting the number of colony forming units and comparing them to the dilution factor. Each colony forming unit represents a bacterium and fungus that was presented in the diluted sample. The numbers of colony forming units are divided by the product of the dilution factor and the volume of the plated diluted suspension to determine the number of bacteria and fungi per ml that were present in the original solution.

Calculating the number of bacteria per mL of serially diluted bacteria: The number of bacteria and fungi per ml of diluted sample was calculated by using following equation:

$$No.of\ CFU/ml = \frac{Number\ of\ CFU}{\text{Volume plated (mL) x total dilution used}}$$

The calculated colony forming units of *Bacillus thuringiensis* are given in Table 2a, b and c.

Experimental layout: The experiment was laid out in completely randomized design having three repeats under *in vitro* conditions. For each treatment, a 50 mm diameter leaf disc was cut out of a healthy *Brassica* crop and dipped into 5 ml of conidial suspension for 10 seconds while excess suspension was removed by placing the leaf discs on sterile filter paper for few minutes. The control leaf discs were treated with 0.05% Tween 80 only. These discs were then placed on moist filter paper in plastic Petri plates. Healthy aphids were distributed with the camel hair brush per replication on treated and untreated leaf discs and incubated at 23±2°C with a 16:8 L: D. The mortality data were recorded over a period of three days at 12-hour interval.

Table 2a. Calculated colony forming units of *Bacillus thuringiensis.*

Concentrations	*Bacillus thuringiensis* Colony	Calculated CFU
5%	128	1.28×10^7
10%	258	2.58×10^7
15%	390	3.90×10^7
20%	521	5.21×10^7
25%	649	6.49×10^7

Table 2b. Calculated colony forming units of *Beauveria bassiana.*

Concentrations	*Beauveria bassiana* Colony	Calculated CFU
5%	95	0.95×10^8
10%	188	1.88×10^8
15%	286	2.86×10^8
20%	382	3.82×10^8
25%	478	4.78×10^8

Table 2c. Calculated colony forming units of *Metarhizium anisopliae.*

Concentrations	*Metarhizium anisopliae* Colony	Calculated CFU
5%	104	1.04×10^8
10%	210	2.10×10^8
15%	321	3.21×10^8
20%	428	4.28×10^8
25%	539	5.39×10^8

Cadavers were shifted to Petri dishes with moist filter paper to promote fungal development and sporulation in order to confirm that death is due to fungal infection. The same procedure was applied for IGRs with addition of movement of their body appendages like legs and antennae were observed under microscope. The aphids showing no movement of their appendages were considered dead. The dead aphids were counted to calculate percentage mortality.

Statistical analysis: The percentage mortality of insects was calculated by the Henderson and Tiltion formula (Henderson and Tilton, 1995).

$$\text{Corrected \%} = \frac{n \text{ in Co before treatment} \times n \text{ in T after treatment}}{n \text{ in Co after treatment} \times n \text{ in T before treatment}} \times 100$$

RESULT AND DISCUSSIONS

Impact of different concentrations of biopesticides on the mortality of mustard aphid: A significant variation in the mortality of mustard aphids was observed when exposed to various concentrations of biopesticides ($P < 0.05$). In all tested biopesticides, the mortality increased with increasing concentrations and time.

Beauveria bassiana, *Metarhizium anisopliae* and *Bacillus thuringiensis* explained mortality in mustard aphids ranging from 16% to 78%, 19% to 83% and 8% to 73%, respectively, being highest at 25% concentration and lowest at 5% concentration. At highest concentration (25%), maximum mortality of mustard aphids was exhibited by *M. anisopliae* (83%) followed by *B. bassiana* (78%) and *B. thuringiensis* (73%) (Table 3).

The main objective of current studies was to evaluate the comparative efficacy of biopesticides *(Beauveria bassiana, Metarhizium anisopliae,* and *Bacillus thuringiensis)* against mustard aphid *Lipaphis erysimi* Kalt. (Hemiptera: Aphididae) in vitro. The study was carried out during the year of 2016, to square the effect of entomopathogenic biopesticides at different concentrations of 5%, 10%, 15%, 20%, 25% and untreated check or control. The determined quantity of each entomopathogenic biopesticides was mixed in water up to required volume to prepare 5, 10, 15, 20 and 25% dilutions/concentration. Mustard aphids *L. erysimi* was picked from brassica fields. Aphid was kept in ventilated plastic jars, for checking disease and parasitism, healthy individuals was used in pathogenicity assays. Experiment was carried out under Complete Randomized Design (CRD) with three replications of each treatment.

Table 3. Mortality of mustard aphids caused by biopesticides at their various concentrations.

Biopesticides	% Mortality					
	control	5%	10%	15%	20%	25%
Beauveria bassiana	16 a ± 0.96	28 a ± 0.93	36 a ± 1.66	45 a ± 1.92	60 a ± 2.72	78 a ± 3.16
Metarhizium anisopliae	19 a ± 0.88	31 a ± 1.36	43 a ± 2.15	56 a ± 2.88	70 a ± 2.54	83 a ± 3.19
Bacillus thuringiensis	8 a ± 0.66	21 a ± 0.96	33 a ± 1.36	40 a ± 1.66	56 a ± 2.75	73 a ± 3.15

The effect of different biopesticides, the mean percent mortality of mustard aphid after the application of all concentration showed that all the biopesticides at higher concentration (25%) and 72 hours provided the maximum mean percent mortality, *M. anisopliae* (83%) and *B. bassiana* (78%), *B. thuringiensis* (73%) all treatment showed the varying degree of control. According to the above mean percent mortality the *M. anisopliae* (83%) proved most affective against mustard aphid whereas *B. thuringiensis* 73% was least effective showing by the percent mortality and statistically (Figure 1). Similar results were also reported by Ujjan and Shahzad (2012) who reported that *B. bassiana*, *M. anisopliae* have been effective and virulent in controlling the mustard aphid. *B. bassiana* provided the mortality up to 88% after 3 days and *M. anisopliae* 72% at high concentration. The finding revealed that entomopathogenic fungi had potential to reduce aphid population from filed.

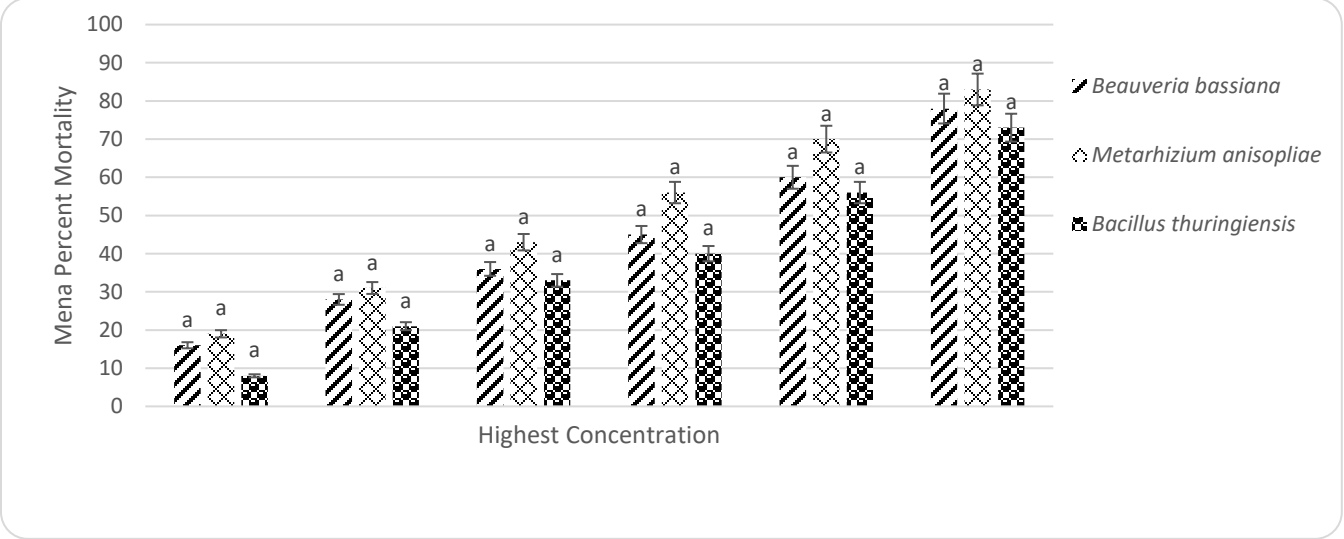

Figure 1. Mean percent mortality of mustard aphid at highest concentration of biopesticides at different time interval (bars showing similar letters in same case differ significantly from each other at probability value of 0.05).

Araujo et al. (2009) has reported 90% mortality with high concentration (10^7 spore per ml) of *B. bassiana* after 4.4 days, while the present study provided mortality 78% after 3 days with high concentration of *B. bassiana* (25%).

Suresh et al. (2012) recorded percent morality with 12-hour interval up to seven days and concluded that mortality of aphid increased with the increase in concentration, at high concentration the mortality was obtained after 72 hours was ranging between 53 to 60 percent, however in current study mortality was recorded up to 78% of *B. bassiana* at high concentration and similarly in case of *M. anisopliae* aphid mortality was obtained 60 to 70% while in present study the mortality was 83%. Loureiro and Moino (2006) reported 100% mortality of turnip aphid through *M. anisopliae* and *B. bassiana* at 10^7 and 10^6 spores/ml respectively. According to Ahmad et al. (2007) least mortality of aphid was monitored in the treatment of BtA after 48 and 72 hours of application of treatment. BtA reduced aphid population by 70% while in current study the mortality of aphid was observed 73% after 72 hour of application of *B. thuringiensis* treatments.

On the basis of above discussion, it may be suggested that the best insecticide, can be used against mustard aphid. On the numerical basis however, among biopesticides *M. anisopliae* was found most effective than *B. bassiana* and *B. thuringiensis*. Biopesticides can be promising and alternate contestant against chemical pesticides in integrated pest management with less chance of insect resistance development, health and environmental hazard and beneficial fauna.

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